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LAMBDA CHAINS AND GENES IN INBRED MICE

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INTRODUCTION

In inbred mice, immunoglobulins (Igs) with κ light (L) chains outnumber those with λ L chains by about 20:1 (1–3). A similar or even greater disproportion is found in mouse myeloma proteins (4). Therefore, amino acid sequence analyses of Ig L chains were initially carried out on κ chains. Many Vκ sequences, but only a single Cκ sequence, were revealed, in accord with later evidence for many $V\kappa$[1] but only a single $C\kappa$ gene segment (5, 6). Amino acid sequences of λ chains made by myeloma tumors suggested a similar arrangement, except that these sequences could be accounted for by only a single $V\lambda$ gene, as well as a single $C\lambda$ gene (7). The extremely limited sequence variability of the λ chains [see (7)] suggested, moreover, that they were not significant contributors to the enormous structural and functional diversity that characterizes murine Igs as a whole. While this viewpoint has not been altered by the subsequent finding of additional λ chain isotypes[2] (8–10), the overall theme of this review is that the very simplicity of the inbred mouse λ light-chain system offers special opportunities to learn about some fundamental properties of Igs, namely, structure-function relationships, Ig diversity, and regulation of Ig gene expression. This system may also turn out to contribute to our understanding of the T-cell antigen receptor, since recent findings point to significant structural homologies between gene segments for λ chains and for some receptor subunits (111, 112).

[1] In accord with convention, genes are italicized and their products are in roman type.

[2] Nomenclature: By convention the variant forms of an immunoglobulin chain are usually called isotypes when encoded by nonallelic genes. Accordingly, we refer to in this paper the three kinds of λ chains as isotypes, though elsewhere they are often called subtypes.

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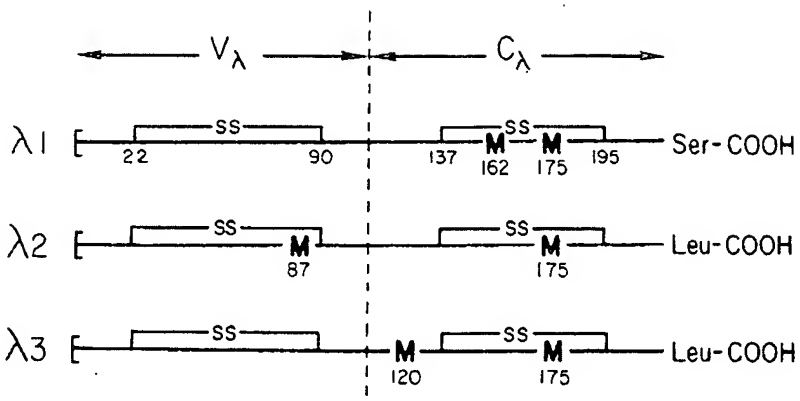

Figure 1 The distinctive positions of methionine residues (M) in each of the three λ chain isotypes. The positions of methionine and half-cystine residues are based on (8–11) and (20).

THE THREE λ CHAIN ISOTYPES

The most frequently encountered λ chain isotype (λ1) was found by analyzing the L chains produced by myeloma tumors that were unselected except for their production of λ1 chains (11–14). The second isotype (λ2) was revealed by the sequence of an L chain (8, 9) that was analyzed because it was from the first myeloma protein (MOPC-315) that was found to have high affinity for commonly studied ligands (DNP, 2,4-dinitrophenyl, and TNP, 2,4,6-trinitrophenyl) (15). Because of its similarity to λ2, the third type (λ3) was discovered (10) while analyzing L chains that were initially considered to be of the λ2 variety (16).

The three isotypes are compared diagrammatically in Figure 1. Each is defined by a distinctive C-region sequence (Figure 2) (9–11). Cλ2 and Cλ3 differ at only 5 out of 104 positions and have not been consistently distinguished serologically; however, they differ markedly from Cλ1 in sequence (Cλ2 at 39 and Cλ3 at 40 residues, see Figure 2) and they are easily distinguished from Cλ1 serologically.

The V region of each λ chain (positions 1–110),[3] like that of each κ chain, is encoded by separate *V* and *J* gene segments that become linked during B-cell development by a rearrangement that joins a *V* to a *J* segment (17). The

[3] Numbering system: All sequenced murine λ chains have the same number of V region amino acid residues. In this paper the positions are, accordingly, numbered sequentially, 1–110, from the amino-terminus to the end of the J region. The numbering is not interrupted by the letter designations introduced by Kabat and Wu (for residues in the first and third CDR) to maximize sequence homology of V regions of H, κ, and λ chains (41). As a consequence of this difference the λ chain positions designated here as 96, 97, 98, and 99 correspond to 94, 95, 96, and 97, respectively, in the Kabat-Wu numbering system (41).

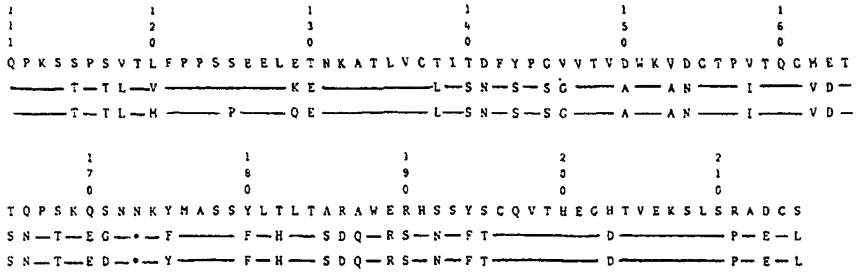

Figure 2 Amino acid sequences of the constant (C) regions of the three λ chain isotypes. The λ1 constant region contains one more residue than the λ2 and λ3 constant regions. The difference is introduced at position 173 to maximize sequence homology (10). Amino acids are designated by the one-letter code. Same residue as λ1 : ——. Based on (8–11).

*V*λ segments encode amino acid positions 1–96 and the *J*λ segments encode positions 99–110. Positions 97 and 98 mark the V/J boundary: As discussed below, nucleotides for the codons that specify the amino acids at these positions can be derived from *V*λ or *J*λ gene segments or both [see (18, 19)].

One of the differences between λ and other Ig chains (κ, H) arises from the invariant association of particular *J*λ and *C*λ segments. A hint of this invariance emerged when the first λ2 and λ3 chains sequences (9, 20) were compared with the previously determined sequence for λ1 chains (11): The C region of each isotype appeared to be associated with a different J sequence (Figure 3). The significance of this association became evident when the genomic organization of λ gene segments was clarified.

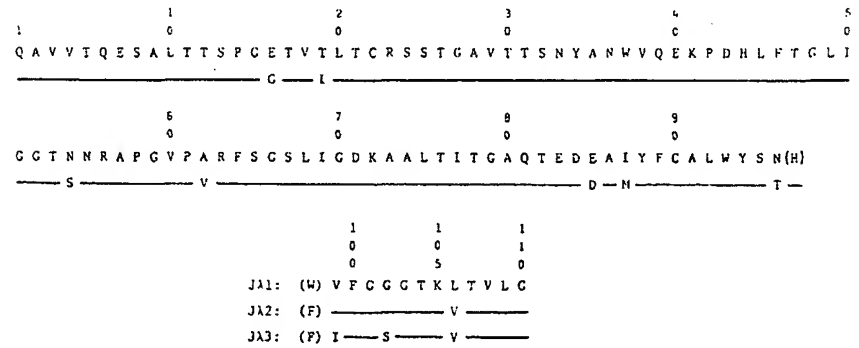

Figure 3 Amino acid sequences of the variable (V) regions of the three λ chain isotypes. The sequences shown correspond to the two germ line *V*λ gene segments (Vλ1, Vλ2) and to the three germ line *J*λ gene segments (Jλ1, Jλ2, Jλ3). Parentheses are placed around the amino acids at positions 97 (His) and 98 (Trp or Phe) to indicate that imprecision in joining *V* to *J* gene segments can result in various amino acid substitutions at these positions (see Figure 5). Same as Vλ1 or Jλ1 : ——. Based on (8–11, 18, 20, 21, 25, 26, 43, 110).

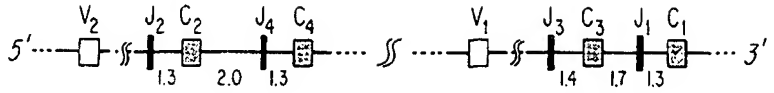

Figure 4 Organization of λ gene segments on chromosome 16 of the BALB/c mouse. The two clusters of *J-C* segments have not been linked physically to each other or to the *Vλ* gene segments. Distances between *Jλ* and *Cλ* segments are in kilobases. The basis for the 5′–3′ order is discussed in the text. Based on (23, 24, 29).

ORGANIZATION OF λ GENE SEGMENTS

Tonegawa et al discovered that there are two *Vλ* gene segments [*Vλ1*, *Vλ2*; see Figure 3 and (21, 22)]. Later, studies by Blomberg et al (23) and by Miller et al (24) showed that the *Jλ* and *Cλ* gene segments are distributed between two similar clusters, arranged as illustrated in Figure 4. Each cluster contains two *Cλ* gene segments, each separated on its 5′ side by a 1.3–1.4 1 kb (kilobase) intron from a unique *Jλ* gene segment. As a result there are four distinct *Jλ-Cλ* pairs. Since each λ isotype is defined by a unique *C* gene segment (Figure 2) and each *Cλ* segment is associated with a particular *Jλ* gene segment (Figures 3, 4), each λ isotype is also characterized by a distinctive J+C sequence, extending from position 99 to the carboxy terminus. The λ1, λ2, and λ3 isotypes are thus encoded, respectively, by the *Jλ1-Cλ1*, the *Jλ2-Cλ2*, and the *Jλ3-Cλ3* gene-segment pairs. The fourth pair, *Jλ4-Cλ4*, has several anomalies, suggesting that it is a pseudogene (25, 26). For example, *Jλ4* lacks a GT dinucleotide that occurs at codon 110 in all functional *J* gene segments and is an obligatory signal element for RNA splicing (27). *Jλ4* also has a 2 bp deletion from the heptamer sequence that serves as a signal for *V-J* rearrangement (-- CAGTG in *Jλ4* versus CACAGTG in *Jλ1*). These anomalies may explain why a λ4 chain has not been encountered and why even a nonproductive *Vλ* rearrangement to *Jλ4* has not been seen (28).

The finding that both rearranged and unrearranged forms of both *Vλ1* and *Vλ2* gene segments can occur in the same cell line [MOPC-315, and J558, (23)] led to the suggestion that *Vλ1* is upstream of the *J3C3-J1C1* cluster and *Vλ2* is upstream of the *J2C2-J4C4* cluster. The 5′–3′ order of these clusters is discussed below. Their localization on chromosome 16 has been demonstrated by Southern blot analyses of mouse-hamster hybrid cell lines (29).

V → J REARRANGEMENTS

A *Vλ* gene segment is not transcribed unless joined to a *Jλ* gene segment. Given two *Vλ* and four *Jλ* segments, eight V-J rearrangements are possible

(22, 23, 28, 30). However, in 74 λ chains whose *V-J* rearrangements were determined directly by Southern blot analyses of myelomas or hybridomas, or deduced from amino acid or cDNA sequences, only three of the eight possible rearrangements occurred regularly (V1-J1, V2-J2, V1-J3). Two occurred rarely (V2-J1, V2-J3), and three were not seen at all (V1-J2, V1-J4, V2-J4) (28). In this series, the *Vλ1* gene segment was recombined with *Jλ1* in nearly all (95%) λ1 chains and with *Jλ3* in nearly all (95%) λ3 chains, whereas *Vλ2* was recombined with *Jλ2* in all λ2 chains examined. In three rare hybridomas, *Vλ2* was recombined with *Jλ3* once (28, 31) and with *Jλ1* twice (28); in the resulting rare chains the encoding gene segments were *V2J3C3* or *V2J1C1*. These exceptions aside, the rule is that λ1 chains are encoded by *V1J1C1* segments, λ2 chains by *V2J2C2* segments, and λ3 chains by *V1J3C3* segments.

The sharply restricted pattern of *V-J* rearrangements suggests the gene order shown in Figure 4 (28, 31). Given this order, and the model for *V-J* rearrangement in which DNA between the recombined gene segments is deleted (32), it appears that when a λ-producing B-cell develops from a precursor pre-B cell, a *Vλ* gene segment will nearly always rearrange with a *Jλ* gene segment in the nearest downstream *J-C* cluster. Rarely, *Vλ2* rearranges to a *Jλ* segment in the distant cluster, but rearrangement in the upstream direction (*V1-J2*) has not been seen and may not be possible.

Since there is virtually only one *V-J* rearrangement per λ chain isotype, *V-J* combinatorial variation contributes almost nothing to the diversity of λ chains, unlike its role in the diversity of κ and H chains (32–35). With κ, for example, there are four functional *Jκ* (32, 34) and on the order of 200 *Vκ* gene segments (17, 36, 37). If their rearrangements are unrestricted, as is generally believed, there would be 800 Vκ-Jκ combinatorial variants; with H chains the number of such variants must be far greater, because as many as 10 to 20 *DH* segments are additionally recombined in forming *VH-DH-JH* assemblies (38–40).

V/J JUNCTIONAL VARIANTS

Combinatorial variation in the assembly of gene segments (*V-J*, *V-D-J*) can contribute to diversity of V region primary structure not only directly, but also indirectly, because imprecision in assembling the segments can create novel codons at the joining sites. Such V/J junctional variants have been described for κ chains (32, 34, 35). Do they also occur with λ chains?

Until recently (19), all sequenced λ chains had tryptophan (Trp) at position 98 of λ1 chains (7, 41), phenylalanine (Phe) at position 98 of λ2 chains (42); and histidine (His) at position 97, regardless of λ isotype (41, 42). It seemed, thus, that λ chains might lack V/J junctional diversity (as well as V-J

combinatorial variability, see above). However, nucleotide sequences at the 3′ end of unrearranged $V\lambda$ gene segments and at the 5′ end of unrearranged $J\lambda$ gene segments suggest that in-phase $V\lambda$-$J\lambda$ recombinations can lead to various amino acids at the V/J boundary. Shown here in Figure 5 are three possibilities (Trp, Leu, Phe) at position 98 of λ1 chains, two (Phe, Tyr) at position 98 of λ2 chains, and two (His, Gln) at position 97 of λ3 chains. Of

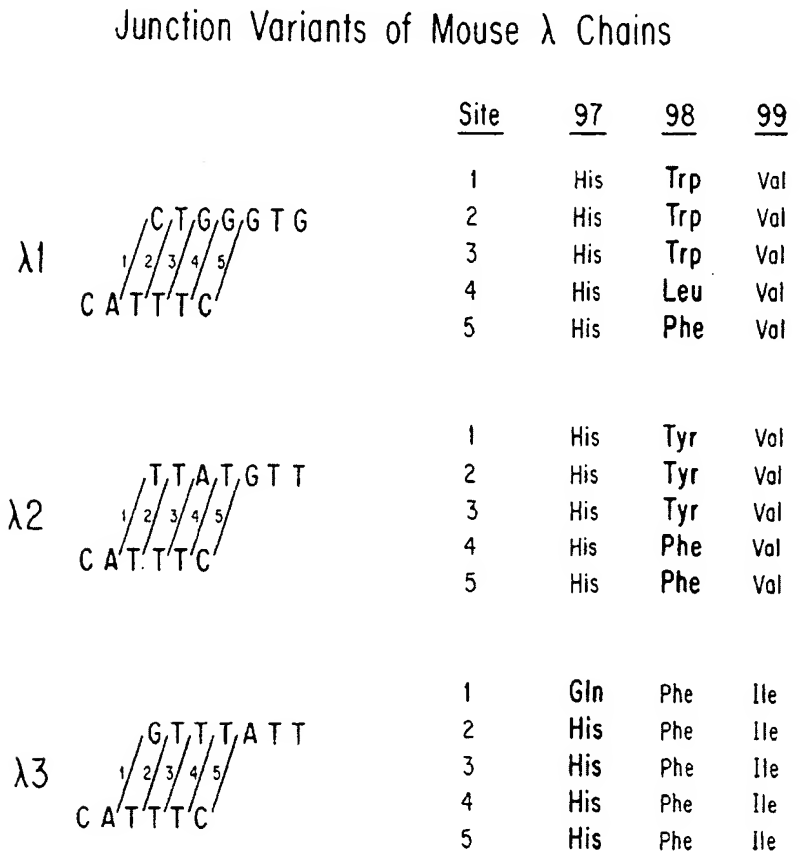

Figure 5 Variation in sites for recombination between $V\lambda$ and $J\lambda$ gene segments. A different combination of segments is utilized for each of the three λ isotypes. For λ1, λ2, and λ3 the recombining segments are (rare exceptions aside), Vλ1-Jλ1, Vλ2-Jλ2, and Vλ1-Jλ3. For each recombining pair shown, the V segment is below and to the left, the J segment is above and to the right; the lines joining them, numbered 1–5, refer to alternative, in-phase recombination sites that link the 3′ end of the *V* segment to the 5′ end of the *J* segment. (Each short sequence is written with 5′ at the left and 3′ at the right.) The resulting alternative amino acids at positions 97–98 are listed at the right bold face. All of the substitutions except Phe at λ1 98 have been observed (18, 19, and footnote 4). The sequences are from (21, 25, 26, 43).

these seven possibilities, six have been detected in recent studies that focussed on λ chains from Igs with unusual ligand binding activity (see STRUCTURE-FUNCTION RELATIONSHIPS). In these studies, a $\lambda 2$ chain with Tyr at position 98 and a $\lambda 3$ chain with Gln at position 97 were identified (18, 19, 42). In an analysis of λ chains from anti-DNP monoclonal antibodies (mAb) from hyperimmunized mice, $\lambda 1$ chains with Leu at position 98, in place of Trp, have also been identified (K. Tamoto, E. B. Reilly, T. Azuma, H. N. Eisen, manuscript in preparation). All of these substitutions at positions 97 ($\lambda 3$) and 98 ($\lambda 1$, $\lambda 2$) can be accounted for by variability in the precise internucleotide site of V-J recombination (Figure 5). Somatic mutation cannot be totally ruled out as an alternative explanation but it is most unlikely, especially when a chain's entire V+J sequence corresponds precisely to the germ-line sequence of the $V\lambda$ and $J\lambda$ gene segments that encode that chain; such a correspondence has been found for all six of the V/J junctional variants identified (18, 19). The special functional significance of amino acid substitutions at the V/J junction is discussed below (see STRUCTURE-FUNCTION RELATIONSHIPS).

V REGION SOMATIC MUTATIONS

Nucleotide or amino acid differences between a germ-line sequence and its expressed V+J sequence are defined as a V region somatic mutation.[4] Therefore, to establish that a somatic mutation has occurred requires that the sequence of the particular germ line V and J segments that encode an expressed V+J sequence be known. Since the nucleotide sequences of all germ line $V\lambda$ and $J\lambda$ gene segments have been established for the BALB/c mouse (21, 25, 26, 43), and the segments that encode any particular λ chain are readily identifiable, the recognition of somatic mutations is straightforward from the $V+J$ sequences of λ chains of BALB/c origin. (For κ and H chains, however, there are many similar $V\kappa$ and many similar VH gene segments; thus it is not easy to identify with certainty the germ line V segments that correspond to V segments that encode particular κ and H chains.)

In an extensive study of V regions of $\lambda 1$ chains, Weigert & Cohn and their colleagues found that 12 of 18 chains had the same V region sequence and that in the other 6 amino acid replacements totalled 7–9 [Figure 6 and (7)]. They correctly inferred that the identical repeats represented the sequence of a $V\lambda$ germ line gene; thus the frequency of V region amino acid replacements (i.e. somatic mutations) in this series of $\lambda 1$ chains was only 0.5

[4] It is formally possible that what appears to be a somatic mutation could be a germ line mutation, i.e. a difference between the consensus germ line sequence for the species or strain and the germ line of the particular animal that produces the expressed V+J sequence.

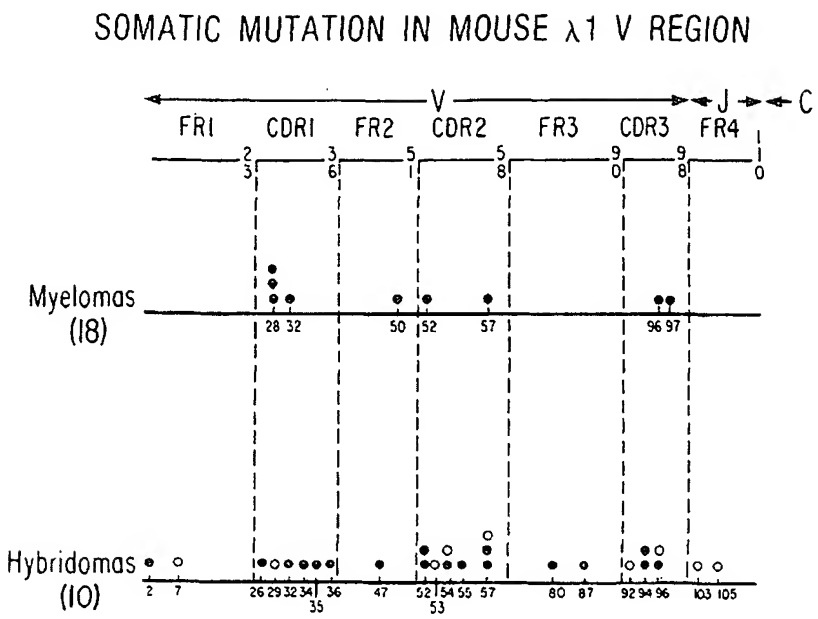

Figure 6 Somatic mutations in V regions of $\lambda 1$ chains. The positions of mutations are shown in chains from 19 myeloma proteins (*top*), analyzed by amino acid sequencing (7), and from 10 monoclonal antibodies (*bottom*) determined by sequencing cDNA from one hybridoma making an IgG2a anti-NP antibody (113), two making IgG1 anti-dansyl and seven making IgG1 anti-DNP antibodies (K. Tamoto, E. B. Reilly, T. Azuma, H. N. Eisen, in preparation). ○, silent mutations, ●, replacement mutations. The V/J junction residues at positions 97 and 98 are not shown.

(or less) per chain. A similar frequency probably exists among $\lambda 2$ chains: In one set of four such chains (42) there was only one V region replacement mutation (0.25 per chain), whereas in another $\lambda 2$ chain [from a myeloma protein with high affinity for DNP ligands (15)] four V-region mutations were found (9), bringing the total for the five $\lambda 2$ chains to five amino acid replacements.

To determine whether the frequency of V region somatic mutations is higher in antibodies from intensively immunized mice than in myeloma proteins, Tamoto et al recently analyzed V regions of $\lambda 1$ chains from hybridomas making monoclonal antibodies (anti-DNP) of the IgG1 class (K. Tamoto, E. B. Reilly, T. Azuma, H. N. Eisen, manuscript in preparation). The choice of these hybridomas was based upon two considerations: (*a*) that the frequency of V region somatic mutations is higher in γ than in μ chains (38, 44, 45–48) and (*b*) that when somatic mutations occur in a B-cell's H chain they are also likely to occur in the V region of the same cell's L

chain. Accordingly, the cDNA from λ1 mRNA was sequenced [by the primer extension method (18)], using as a source of mRNA the hybridomas that were derived from hyperimmunized BALB/c mice and were making IgG1, λ1 monoclonal antibodies (K. Tamoto, E. B. Reilly, T. Azuma, H. N. Eisen, manuscript in preparation). The V region of 10 of these λ1 chains had 18 replacement somatic mutations, or an average of 1.8 per chain (Figure 6). There were also nine "silent" mutations, bringing the average to a total of 2.7 mutations per chain. That one third were silent is expected from the genetic code. The higher frequency of replacement mutations found in this study than in the others probably reflects selection by antigen during hyperimmunization of particular B cells, probably those producing Igs with high affinity for the DNP group (49).

The Mutation Frequency in Complementarity-Determining Versus Framework Regions

The distribution of mutations between complementarity determining (CDR) and framework regions (FR) also suggests that selective pressures influence the nature and frequency of the mutations identified in Igs. In V regions of the sequenced κ chains this distinction is not so clear, as mutations seem to be almost as frequent in FR as in CDR (50–55). However, in the study by Tamoto et al of λ1 chains from IgG1 mAbs derived from hyperimmunized mice, the frequency of replacement mutations was about 20 times higher in CDRs than in FRs (Figure 6) (K. Tamoto, E. B. Reilly, T. Azuma, H. N. Eisen, manuscript in preparation). The low frequency of replacement mutations in FRs suggests that there is strong selection against amino acid substitutions in these regions, probably to conserve their secondary structure and preserve the L-H chain interactions needed to maintain the integrity of the Ig molecule's architecture and its combining sites. In contrast, the high frequency of replacement mutations in CDRs (20 in 270 or almost 1 in 10 codons, see Figure 6) suggests that in these regions there are very few constraints; indeed, CDR mutations that result in better binding sites and higher affinity are probably selected for by antigen.

Number of Primary Structure Variants of λ1 Chains

How many V region variants are there among the three λ chain isotypes? *Vλ1* can recombine with *Jλ1* and (rarely) with *Jλ3*, and each of these rearrangements can result in 2 or 3 V/J junctional variants (neglecting a third possibility, Phe, that has so far not been seen; Figure 5). There are thus 4 or 5 λ1 and λ3 V/J variants; similarly there are two λ2 V/J variants, each resulting from the *Vλ2-Jλ2* rearrangement. There are also four additional very rare variants due to the unusual *V2-J1* and *V2-J3* rearrangements and

the probability that each of these is also associated with two V/J junctional variants (Figure 5). Hence, altogether, in the absence of somatic mutations, 11 primary structure V region variants are possible: Eight of these have been detected, but three of the 11 probably account for nearly all of the λ chains having germline (i.e. nonmutated) V region sequences: $\lambda 1$ chains having Trp at position 98; $\lambda 2$ having Phe at position 98, and $\lambda 3$ having His at position 97).

However, the contributions of V/J junctional variation (and of V/J combinatorial variation) to λ chain diversity is almost insignificant when compared to the effects of somatic mutation. In calculating the contribution of somatic mutation to diversity we consider that: (*a*) The average single base change that leads to an amino acid replacement can occur in the first or second position of a codon and result (on average) in six alternative replacements for the amino acid at that position in the germline sequence; (*b*) these replacement mutations are largely limited to the 27 positions in the three CDRs, since elsewhere in the V region (i.e. in FRs) they are likely to result in an unstable Ig structure; (*c*) the replacement mutations can probably occur in any CDR position, and in these positions there are very likely to be no forbidden substitutions; (*d*) fluctuations in the number of mutations per chain can be estimated from the Poisson distribution. To emphasize the last point we note that under conditions where there are, on the average, 1.8 replacement somatic mutations per $\lambda 1$ chain (see Figure 6), 16% of the chains will have three such mutations, 7% will have four, etc.

Since there are $n!/(n-x)!x!$ different combinations of x replacement mutations in a chain having n susceptible codons, the number (N) of different amino acid sequences is:

$$a^x \cdot \frac{n!}{(n-x)!x!},$$

where a is the number of alternative amino acids resulting from a single base change in the first or second position of a mutated codon, x is the number of such mutated codons per chain, and n is the number of codons that are susceptible to these replacement mutations. For reasons given above, we assume $a = 6$ and $n = 27$. Hence, when x is 0, 1, 2, 3, 4 the corresponding number of chains with different V region amino acid sequences is (respectively) 1, 162, 1.26×10^4, 6.26×10^5, and 2.28×10^7. To account for V/J junction variation each of these numbers should be multiplied by two or three (see Figure 5).

From the findings of Tamoto et al and the foregoing estimates it follows that in BALB/c mice the number of different V-region sequence variants of just this one isotype ($\lambda 1$) is close to and probably exceeds the total number of B-cell clones in the individual mouse. Hence the size of the potential Ig

repertoire in an individual is probably fixed not by its ability to generate Igs with a vast number of V region sequences but rather by the total number of B-cell clones it has and the rate at which these clones turn over, i.e. are replaced by new clones, arising from precursor cells.

STRUCTURE-FUNCTION RELATIONSHIPS

A central dogma of immunology is that amino acid sequences in CDRs determine an antibody molecule's specificity and affinity for ligand. However, the application of this generalization to particular sequences and ligands has been almost impossible, and the general rules that govern the connection between particular primary structures and ligand-binding activities remain almost totally obscure.

Primary Structure and Affinity

Recently, Azuma et al (19) have taken advantage of some currently available λ chains that differ from each other in sequence by only one or a few amino acids to ask whether these few differences affect ligand-binding activity. To answer the question they recombined each of these chains with the H chain (H^{315}) from an anti-DNP myeloma protein (M315) and thereby obtained a set of reconstituted Igs that also differed in sequence by just one or a few amino acids at known positions in the L chains. Differences in affinity of these reconstituted Igs for DNP ligands, summarized in Figure 7, revealed a surprisingly pronounced effect of amino acids at the V/J junction (positions 97 and 98 in λ chains).

In a striking demonstration of this junction effect, two reconstituted Igs (H^{315}-L^{8-13} and H^{315}-L^{5-7}) that differed almost 1000-fold in affinity for DNP aminocaproate differed in primary structure by only a single phenylalanine (Phe)-tyrosine (Tyr) substitution at the V/J junction of their L (λ2) chains (see Figures 8 and 5), i.e. by only one out of approximately 660 amino acids (L + H chains), and, indeed, by only the single O atom difference between Phe and Tyr. In another example, a pair of reconstituted Igs (H^{315}-L^{5-8} and H^{315}-L^{C49}) that differed in sequence by only a glutamine (Gln)-histidine (His) substitution at the V/J junction of their L (λ3) chains (see Figures 8 and 5) also differed in affinity, though only four-fold. If the V/J junction effect proves to be a general one, it would mean that gene segment assembly (V/J in L chains and perhaps also V/D/J in H chains) is an important source not only of primary sequence variation (as is well known) but also of ligand-binding diversity.

If the effect of the V/J junction residues is due to VL interactions with VH, rather than to direct interactions with the bound ligand itself, the impact of a particular residue at the λ chain junction must also depend upon the VH

L Chain		Variable Region Gene Segments Vλ	Jλ	Sequence Differences Between λ Chains and Germline Forms of Vλ and Jλ Gene Segments	Affinity* L/M x10⁶
				Vλ — Jλ 1 16 19 54 62 85 87 96 97 98 99 100 110 Vλ1 E T N A E I N V G L Jλ1 Vλ2 G I S V D M T V G V Jλ2 I S V Jλ3	
λ1	HOPC-1	1	1	H W	<0.005
	H2020	1	1	① ⑤ H W 26 30	<0.005
λ2	8-13	2	2	H F	11.0
	T952	2	2	H F ① 99	6.3
	M315	2	2	① H F 38 94 95 96	6.7
	5-7	2	2	H Y	0.05-0.1
λ3	5-8	1	3	H F	5.0
	5-5	1	3		5.3
	C49	1	3	Q F	1.3
	8-47	1	3		1.5
	6-2	2	3	H F	3.5

* for DNP-aminocaproate; 20°

Figure 7 Correlation between V region sequences of λ chains and affinity for ε-DNP-aminocaproate of the reconstituted Igs made with each of these chains and H^{315}, the heavy chain from an anti-DNP myeloma protein (MOPC-315). Circled amino acid residues indicate differences from the germ line sequences. Comparison of germ line sequences at the top of the Figure shows only those positions and residues at which the *Vλ1* and *Vλ2* and the *Jλ1*, *Jλ2*, and *Jλ3* gene segments differ. From Azuma et al (19).

sequence of the paired H chain. This argument is supported by the observation that although λ chains with junctional Phe, and not with junctional Tyr or tryptophan (Trp), form good DNP-binding sites with H^{315} (see Figure 7), in other Igs, having other H chains, λ chains with Tyr or Trp at the junctional position can be present in Igs with high-affinity anti-DNP sites (for example, the antibody from which L^{5-7} was isolated; also K. Tamoto et al, in preparation).

In contrast to the striking effect of substitutions at the V-J boundary, amino acid substitutions elsewhere in the V region, whether in framework (FR) or complementarity-determining regions (CDR), had little effect on affinity for DNP ligands of the reconstituted Igs referred to in Figure 7. For

instance, a pair of reconstituted Igs (H^{315}-L^{315} and H^{315}-L^{952}) that differed at five V region positions, including three in the third CDR, had essentially the same affinity (6.3×10^6 vs 6.7×10^6 L/M). The lack of effect of these substitutions on affinity for ε-DNP-aminocaproate could mean that this hapten was an inappropriate ligand, perhaps because it is too small (56), or because a DNP-like antigen was not involved (in vivo) in the development of the B cells that gave rise to the myeloma tumors (MOPC-315 and TEPC-952) from which these L chains were derived. This last possibility emphasizes the need for what might be called prospective rather than retrospective analyses. The work of Azuma et al (19), summarized in Figure 7, represents a retrospective study: Myeloma proteins and monoclonal antibodies that happened to be available were used to furnish the chains for analysis of structure-function relationships with a ligand (DNP) that had no clear relationship to the antigenic stimulus that elicited the production of most of these Igs. It may prove more informative to carry out future studies of the type illustrated in Figure 7 with chains from monoclonal antibodies that have been deliberately elicited by an antigen that resembles the ligand used to analyze the reconstituted Igs.

Some λ chains, in the absence of H chains, bind to ε-DNP-lysine Sepharose beads (19). Free λ chains are predominantly dimers in solution and the L^{315} dimer is known to bind DNP-lysine (57, 58), though with several orders of magnitude lower affinity than the corresponding intact Ig (M315) or reconstituted Ig (H^{315}-L^{315}; see Figure 7). In general, the binding of various L chains to ε-DNP-lysine beads paralleled differences in affinity of the reconstituted Igs formed by the same L chains with H^{315} (19).

Primary Structure and Idiotypy

Because of their limited sequence diversity, λ chains are also useful for studying the relationship between primary structure and idiotypy. To illustrate this point, consider the reconstituted Igs made with H^{315} and the λ chains shown in Figure 7. These Igs were tested for ability to block the binding of ^{125}I-M315 by antibodies to the idiotype (Id) of myeloma protein 315 (M315). (The anti-Id antibodies were produced by immunizing BALB/c mice with M315, a protein of BALB/c origin; the resulting isologous anti-Id antibodies reacted only with M315, not with other myeloma proteins (59, 60).) Only the homologous reconstituted Ig (H^{315}-L^{315}) blocked this reaction, and it did so completely and as well as native M315 in inhibition-titration assays; the other reconstituted Igs, shown in Figure 7, were without any effect (T. Azuma, V. Igras, H. N. Eisen, manuscript in preparation).

What distinguishes the primary structure of L^{315} from that of other λ2 chains are the four circled amino acids shown in Figure 7. One, a

conservative substitution in the second FR, is probably insignificant and it is very likely that the three contiguous substitutions in the third CDR, Phe-Arg-Asn, are required for the Id that is recognized by isologous anti-315 Id antibodies.

Idiotypic determinants recognized by anti-Id antibodies depend not only on L, but also on H chains (59). Thus, reconstituted Igs made with L^{315} and H chains other than H^{315} also do not react with isologous anti-M315 (Id); therefore this Id is defined by some as-yet-unknown sequence in the V region of H^{315}, in addition to the third CDR Phe-Arg-Asn of L^{315}.

The isologous anti-Id responses elicited by myeloma protein M315 in BALB/c mice involve not only antibodies but also T cells. Some of these cells cause a delayed type hypersensitivity (DTH) reaction and others seem to suppress this reaction (66). Unlike the isologous anti-315 Id antibodies, the isologous anti-315 Id T cells seem to react with L^{315} alone, in absence of H chains, but they do not react with other λ chains. Very likely, therefore, the amino acids that are necessary, and perhaps sufficient, for recognition by the isologous anti-315 Id T cells are also the three contiguous products of somatic mutation in the third CDR of L315 [Phe-Arg-Asn, Figure 7; (66)].

FREQUENCY OF λ CHAINS AND λ ISOTYPES

λ-B Cells vs κ-B Cells

In accord with the much greater abundance in serum of κ-Igs than λ-Igs, κ-producing B cells are over 20-times more numerous than λ-producers in LPS-stimulated spleen cells (67–69). Interestingly, however, in similarly stimulated bone marrow cells κ-producers are only about four times more frequent than λ-producers (69). In bone marrow the ratio of κ-: λ-B cells (4:1) probably represents B cells that have just arisen from pre-B cells, whereas in spleen the ratio ($>20:1$) probably reflects antigen-selection of mature B cells, with greater stimulation of κ-B than λ-B cells by randomly encountered antigens, because V region diversity of surface Igs of the κ-B cell population must greatly exceed that of the λ-B cell population.

It would be useful to know the frequency in bone marrow of B cells that produce each of the λ isotypes. In the absence of this information the frequencies of the λ isotypes in Igs and in B cells, described below, probably reflect the effects of antigen-selection, as much as (or perhaps even more than) the intrinsic frequencies in virgin B cells.

Normal Serum Ig

In sexually mature mice the average serum concentrations of Igs with $\lambda 2$ chains ($\lambda 2$-Igs) plus those with $\lambda 3$ chains ($\lambda 3$-Igs) varied among the inbred

strains from a high of about 80–140 μg/ml in the AL/N, NZB, BALB/c strains to a low of about 15–25 μg/ml in the BSVS, C58/J, CE/J, SJL/J, DBA/2, and C3H/He strains (70). The differences did not correlate with Ig heavy chain allotypes or major histocompatibility complex haplotype. In BALB/c mice λ1-Igs are about four times more frequent than λ2-Igs (3), and the latter are probably somewhat more frequent than λ3-Igs (71). Based on these relationships, serum concentrations of λ1-Igs are calculated to be about 300–400 μg/ml, corresponding to an estimated total serum Ig concentration of about 7 mg/ml. In SJL mice (see below), which virtually lack λ1-Igs (72, 73), the level of λ2-Igs (and probably total λ-Ig) is only about 25 μg/ml (70). In mass terms, therefore, λ-Ig represents an almost negligible proportion of total normal serum Ig.

In Antibodies Elicited By Certain Antigens

Despite the low frequency of λ-Ig in normal serum and the paucity of $V\lambda$ genes, certain antigens elicit antibodies that contain a high proportion of λ chains. Two that are known to evoke such responses are the 4-hydroxy-3-nitrophenylacetyl (NP) group (74) and dextrans having an alternating (1–3),(1–6) backbone (75, 76). It has recently been found that high λ-antibody responses are also elicited by the 2,4-dinitrophenyl (DNP) group (77) and by the 5-dimethylaminoapthalene-1-sulfonyl (dansyl) group (K. Tamoto et al, in preparation).

In the primary response to NP-protein conjugates about 90% of the antibodies made in C57BL/6 mice have λ1 chains (74; and T. Imanishi-Kari, personal communication) and in BALB/c mice about 50% have λ1 chains (78). Thereza Imanishi-Kari has also shown, more strikingly, that λ1 chains are present in 49 out of 50 anti-NP monoclonal antibodies from hybridomas generated by primary immunization of C57BL/6 mice; the remaining one had a λ3 chain (T. Imanishi-Kari, personal communication). A useful way to analyze the comparative frequency of λ isotypes in antibodies is illustrated in Figure 8. ^{35}S-methionine-labelled polyclonal anti-NP antibodies made by spleen cells from C57BL/6 mice (immunized with NP-chicken γ globulin) also reveal the great predominance of λ1 over the other isotypes (77).

In the response of BALB/c mice to dextran with the alternating (1–6),(1–3) backbone nearly all the antibodies contain λ chains (75, 76). Their subtypes have not been thoroughly characterized, but from one preliminary observation they seem to be almost exclusively λ1 (V. Igras, personal communication).

A recent study of the L chains of anti-dansyl antibodies by Fan & Karush (79) together with Tamoto, Reilly, Eisen (in preparation) has also revealed in this response a surprisingly high proportion of λ chains. The anti-dansyl

molecules made in BALB/c mice against dansyl-ficoll and in primary and secondary responses against dansyl-bovine γ globulin have L chains that are mainly λ1, but the predominance of this isotype differs somewhat in IgM and IgG molecules. In the IgM anti-dansyl monoclonal antibodies, about 80% had λ chains, but in this set λ1 was no more frequent than the sum of λ2 and λ3. In the corresponding IgG antibodies, about 95% had λ chains and of these about 50% were λ1, 33% were λ2, but none was λ3. The excess of λ1 over λ3 is especially striking and is commented on below.

In antibodies made against the DNP group (another response recently shown to have a high proportion of λ-antibodies (77)), about 40% of the polyclonal anti-DNP antibodies made in primary and secondary responses of BALB/c mice to DNP-chicken γ globulin have λ chains (Figure 9) (77). In 60% of these antibodies the isotype is λ1; the other 40% seem to be divided about equally between λ2 and λ3. Thus in BALB/c anti-DNP molecules λ1 is only about three times more frequent than either of the other λ isotypes. Similar results have been found in (77): (*a*) culture supernatants from about

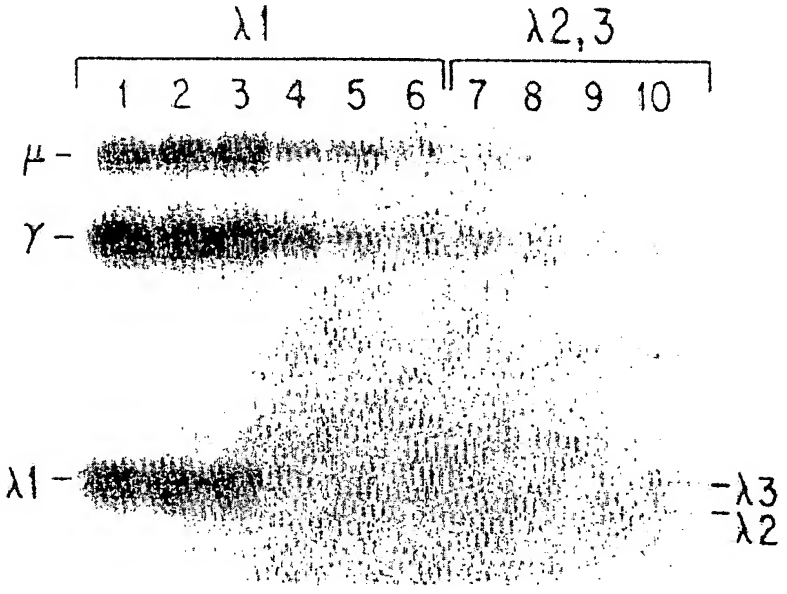

Figure 8 λ chain isotypes in anti-NP antibodies. ^{35}S-methionine-labeled antibodies, synthesized by spleen cells from BALB/c mice immunized with NP-chicken γ globulin were purified, immunoprecipitated sequentially six times by antibodies to λ1 and then four times by antibodies to the other isotypes and analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography. Note that λ1 chains are abundant whereas λ2 and λ3 chains are virtually undetectable. From (77).

200 hybridomas, and (*b*) the ^{35}S-methionine labeled antibodies secreted by spleen cells (Figure 9). Similar frequencies have also been found in the predominantly IgM anti-DNP monoclonal antibodies elicited by DNP-ficoll (80).

It is not clear why λ chains are so prominent in the response to certain antigenic groups. There are no obvious chemical features that distinguish the antigens that elicit predominantly λ-Abs. For instance, one of these is the anionic form of NP; others are hydrophobic and nonionic, and one is a polysaccharide [dextran with the alternating (1,3),(1,6) backbone].

Why is λ1 the dominant isotype? The reasons for the predominance of λ1 over the other λ isotypes in serum Igs and in antibodies to NP, DNP, dansyl, and dextran are also not known. The very large difference between λ1 and λ3 is particularly puzzling, because in the functional (i.e. rearranged) gene for nearly all chains of both isotypes the same Vλ1 gene segment is used (28). Hence, the rearranged λ1 and λ3 genes have not only the same V region sequence from positions 1–98 (ignoring somatic mutations) but also

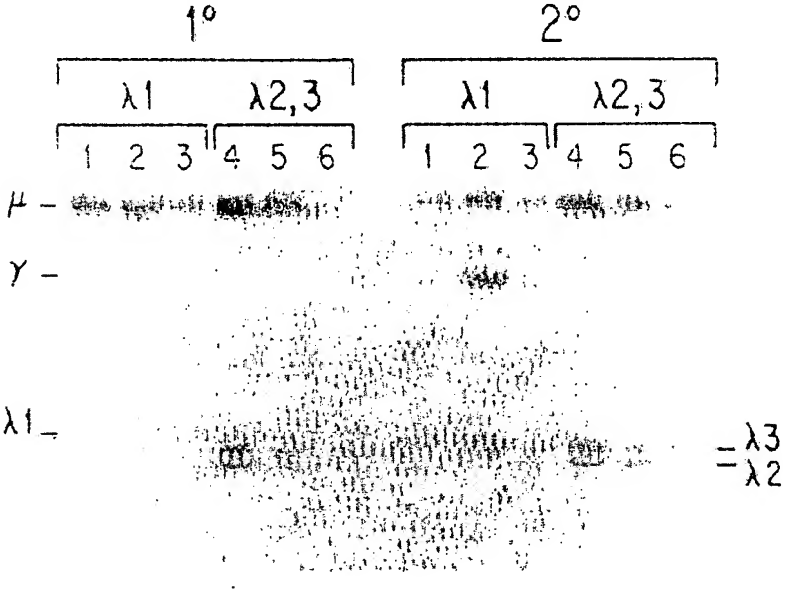

Figure 9 λ chain isotypes in anti-DNP antibodies. ^{35}S-methionine-labeled antibodies, synthesized by spleen cells from BALB/c mice immunized with DNP-chicken γ globulin, were purified after primary, 1°, and secondary, 2°, immunizations and analyzed as described in Figure 8. Note that in these antibodies λ2 and λ3 chains are relatively abundant: λ1 chains are actually slightly more abundant but appear less so here, perhaps because they migrate as less compact bands than the λ2 and λ3 chains. From (77).

the same upstream noncoding sequences for promoter(s) and possible enhancers. Moreover they can have the same V/J junctional residues (Figure 5).

Several possible explanations for the λ1 predominance have been considered. One ("antigen-selection") is that the antibodies formed by particular H chains and λ1 chains might have higher affinity for an antigen than the corresponding molecules formed with λ2 or λ3 chains. However, this explanation seems not to be valid, because no significant differences in affinity for NP-ligands were observed for reconstituted Ig molecules made by recombining various λ1, λ2, and λ3 chains with the H chains isolated from λ1-containing anti-NP monoclonal antibodies (81). Moreover, the reconstituted molecules made with λ2 or λ3 chains reacted with antibody to the NP^b Id, the characteristic idiotype of λ1-containing anti-NP antibodies from C57BL/6 mice (81). This limited observation suggests that idiotype regulation is probably also not responsible for the λ1 predominance.

Isotype-specific regulatory T cells, that might enhance responses of λ1-B cells or suppress responses of λ2-B cells and λ3-B cells also do not seem to be responsible for the markedly different λ-isotype frequencies in the anti-NP and anti-DNP responses. Though λ1 is the predominant L chain isotype in both anti-NP and anti-DNP antibodies, the frequency of λ2 and λ3 chains is greater in anti-DNP than in anti-NP antibodies. Thus, the ratio of λ1/λ2 + λ3 in anti-NP is about 30, whereas in anti-DNP it is about 1.5 (77). This large difference was consistently observed in BALB/c (and C57BL/6) mice injected with NP-chicken γ globulin (to elicit anti-NP antibodies) or DNP-chicken γ globulin (to elicit anti-DNP antibodies). If regulatory T cells were responsible for the isotype disparity between the anti-NP and anti-DNP responses, the injection of a mixture of the two antigens (NP-CGG and DNP-CGG) would be expected to lead either to preferential stimulation of λ1-producing B cells or preferential suppression of λ2- or λ3-producing B cells, with the result that the λ1/λ2 + λ3 ratio in the resulting anti-DNP molecules would come to resemble the ratio in the anti-NP Abs (or vice versa). However, the ratios were unaffected by immunizing the mice with either antigen alone or with a mixture of the two (77).

In Normal (Resting) Spleen B Cells

The predominance of Igs with λ1 chains over those with λ2 or λ3 chains would be understandable if λ1-B cells were much more numerous than λ2- or λ3-B cells. However, immunofluorescence has indicated that λ1- and λ2-B cells have nearly the same frequency in fetal liver and in normal spleen (83). Because the test antiserum probably did not distinguish between λ2 and λ3 this meant that the frequency of λ1-B cells equaled the sum of frequencies of λ2- plus λ3-B cells. In agreement, the ratio of ^{35}S-labeled λ1,

λ2, and λ3 chains synthesized by normal spleen cells, measured by immunoprecipitation and SDS-polyacrylamide gel electrophoresis, was indeed subsequently found to be about 1:0.7:0.3 (71). Virtually all λ chains have two methionine residues per chain (Figure 1), and the rate of incorporation of ^{35}S-methionine into the various λ isotypes was roughly proportional to the frequency of the corresponding B cells. Hence, it is probable that the rate of λ chain synthesis is the same per resting B cell, regardless of the isotype it produces.

The small differences in frequency of various λ-B cells appear not to be due to selective effects of environmental antigens or regulatory T cells, since the same ratios were found in adult spleen and in neonatal and nude mouse spleen (71). It is possible that these differences arise during B-cell development from different probabilities in the V-J joining reaction for the different isotypes. Different heptamer signal sequences at the 5′ end of the three functional *Jλ* gene segments could result in *V-J* rearrangements being more probable for *Vλ1-Jλ1* than for *Vλ2-Jλ2* and least probable for *Vλ1-Jλ3* (25, 26).

In Polyclonally Stimulated Spleen Cells

Despite the small differences in frequency of the various λ-B cells in normal (resting) spleen, when these cells are stimulated by mitogens they produce greatly disparate amounts of λ chain isotypes, more in accord with the frequencies in serum Ig and in various antibodies. In LPS (or 8-bromoguanosine)-stimulated spleen cells, λ1 chains were synthesized 7 times faster than λ2 chains and 10 times faster than λ3 chains (82). When these rates were corrected for the relative frequencies of the respective B cells (which are the same in LPS-stimulated and resting spleen cells) λ1 chains appeared to be synthesized five times faster per stimulated cell than λ2 or λ3 chains; the latter two were made at about the same rate per cell (82).

Are there λ gene enhancer sequences? Why should λ1 chains be produced faster (per B-cell blast) than each of the other λ chains? A possible explanation is suggested by sequence homologies. As noted before, functional (i.e. rearranged) *λ1* and *λ3* genes nearly always have the same *Vλ* gene segment (*Vλ1*) (28) and therefore the same noncoding upstream sequences (for promoters, etc). However, downstream of their respective *V* and *J* gene segments, the rearranged *λ2* and *λ3* genes are very much alike and both differ markedly from *λ1* in *C* gene segments (see Figure 2), probably in the noncoding *J-C* introns (25), and perhaps also in untranslated sequences downstream of the *C* segments. It is possible, therefore, that regulatory sequences in untranslated regions that flank the *C* gene segments (or perhaps even in this segment itself) are responsible for the disparate rates of expression of the rearranged genes for λ1 and the other

isotypes. Though enhancer sequences in the *J-C* introns seem to be required for transcription of rearranged genes for H and κ chains (84–88), the experimental approaches that demonstrated H and κ enhancer elements have not succeeded so far in revealing similar elements in the rearranged *λ1* gene (89).

Nonetheless, there is preliminary evidence for a λ1 enhancer. This evidence derives from gene-transfer experiments using a modified retrovirus vector (90) that contains two exogenous, inserted sequences, one a rearranged *λ1* gene and the other a Neomycin-resistance gene. The resistance gene confers on eucaryotic cells the ability to grow in what are otherwise toxic concentrations of a neomycin-like drug (G418) (91). When various cultured lines of B and T cells and fibroblasts were infected with this modified vector the lymphocytes, but not the fibroblasts, became G418-resistant, providing the resistance gene had a neighboring rearranged *λ1* gene insert (R. Cone, E. B. Reilly, in preparation). These findings suggest that a tissue-specific (i.e. a lymphocyte- or perhaps a B cell–specific) enhancer element in the *λ1* gene facilitates expression of the Neomycin-resistance gene. If further analyses support this view, similar studies with rearranged *λ2* and *λ3* genes may make it possible to determine whether or not these genes have less potent enhancer sequences than *λ1*. It should be noted, finally, that the disparity in isotype expression is evident in LPS-stimulated B cells, not in resting B cells and not, so far as is known, in the most highly differentiated B cells (plasma cells, myelomas, hybridomas). If there are significant differences among the λ-isotype enhancers, there may also be some differences between B-cell blasts and resting or fully differentiated B cells in the cellular proteins that interact with enhancer sequences.

Defective Expression of λ1 Chains in SJL Mice

λ1 chains are barely detectable in SJL mouse serum, the concentration being about 1/30th the level in other strains (72, 73). The segregation of the low serum λ1 phenotype in progeny of crosses between SJL and BALB/c, a strain with normal levels, suggests that the production of these chains is controlled by a single gene, *rλ1*, with two alleles, one ($\lambda 1^+$) associated with conventional levels, the other ($\lambda 1^{lo}$) associated with barely detectable levels (73). The *rλ1* is not linked to the major histocompatibility locus or to the Ig heavy chain locus (73). Instead, the distribution of serum λ1 values among strains of the BALB/cKe × SJL/J recombinant inbred family indicates that the defect in $\lambda 1^{lo}$ is linked to the *λ1* structural gene (92). The latter was originally identified serologically as a *λ1* allotypic marker (93), which corresponds to a recently discovered sequence polymorphism in the *Cλ1*

gene segment (94). From the intermediate serum λ1 levels in F1 progeny of crosses between λ1-low and λ1-high strains it also appears that *rλ1* acts cis to the structural λ1 gene (73).

Though serum λ1 concentrations are extremely low in SJL, it is possible that these mice produce some normal λ1. Thus, adsorption of SJL serum with anti-λ1 antibodies reduces even further the capacity of these serums to compete with authentic λ1-Igs in radioimmunoassays (73). Moreover, when immunized with the particular dextran (alternating (1–3),(1–6) backbone) that elicits in $\lambda1^+$ strains antibodies with a high frequency of λ1 chains and a characteristic shared idiotype (72, 73, 95) SJL mice produce about 100-times lower levels of anti-dextran antibodies (73). However, the small amount they produce does contain λ1 (or λ1-like) chains and the idiotype that is characteristic of anti-dextran in $\lambda1^+$ strains (72, 73, 95). Since, however, the V regions of λ3 and λ1 chains are encoded by the same Vλ1 gene segment (28), it is possible that the SJL Id^+ antidextran antibodies contain λ3 instead of λ1 chains. This possibility can now be readily evaluated (e.g. see Figures 8, 9).

When immunized with a λ1-Ig from a $\lambda1^+$ strain, SJL mice produce antibodies to the C domain of λ1 chains. Indeed, an anti-Cλ1 monoclonal antibody of SJL origin is useful for detecting and measuring concentrations of λ1 (96). The ability to make such antibodies could mean that SJL mice make no normal λ1 chains at all and therefore respond well to immunization with them, or it could mean that the anti-Cλ1 antibodies made in SJL mice are antiallotypic, specific for the limited polymorphic difference between the C domain sequence of $\lambda1^+$ and $\lambda1^{lo}$ strains (see below).

The frequency of λ1-B cells is about 100-times lower in mature SJL than in mature $\lambda1^+$ mice (83). However, the frequency seems to be the same in newborn SJL and in newborn $\lambda1^+$ mice (C57BL/6 and BALB/c). Evidently, with age the frequency increases in $\lambda1^+$ strains but decreases in SJL (83). It is thus possible that the *rλ1* gene controls not so much the number of λ1-B cells that can be formed as the ability of these cells to be stimulated to proliferate and eventually secrete λ1-Igs.

Recently, Arp et al (94) analyzed in DNA from SJL mice the encoding sequences for the *λ1* gene and the nucleotides flanking these sequences, and compared the results carefully with the corresponding sequences in BALB/c ($\lambda1^+$) DNA. The only difference found was one T-G substitution, corresponding to an alteration in the C domain protein sequence at position 155, from a Gly (GGT) in BALB/c to a Val (GTT) in SJL. This nucleotide substitution also results in a restriction enzyme (Kpn)-sensitive site in BALB/c that is lacking in SJL. The Gly/Val substitution could provide the basis for the λ1 allotypic difference between the two strains, and

it could account also for the ability of SJL to form anti-Cλ1 antibodies in response to immunization with λ1-Ig from the C57BL/6 strain (which is a $\lambda1^+$ strain and also has the Kpn-sensitive site in its *Cλ1* gene segment).

Arp et al (94) found no sequence differences between SJL and BALB/c DNA in the heptamer and nonamer signal sequences involved in joining *V* to *J-C* gene segments, or in approximately 100 nucleotides upstream of *Vλ1*, a region that is likely to include the λ1 promoter, or in the noncoding nucleotides downstream of *Jλ1* and upstream of *Cλ1*, which contain sites that are important for RNA splicing. There are thus no grounds for supposing that the *λ1* defect in SJL arises from an ineffective *Vλ1-Jλ1* rearrangement or from abnormal initiation of transcription, or from defective RNA splicing.

It is difficult to see how a single G-T transversion, resulting in a single Gly-Val substitution in the C domain, could have so large an impact on λ1-Ig production. Nonetheless, there is an interesting correlation between this single base change and the λ1 phenotype. The BSVS mouse strain shares with SJL the λ1-low phenotype and the absence of the *Cλ1* Kpn-sensitive site (revealed by Southern blot analyses), whereas a subline of SJL developed by M. Potter has both normal λ1 levels in serum and the *Cλ1* Kpn-sensitive site of the $\lambda1^+$ strains (94).

On balance, however, it seems unlikely that the altered Cλ1 sequence in SJL and BSVS is related to the $\lambda1^{lo}$ phenotype. Indeed, both of these strains also have very low λ2 (plus λ3) levels (70), and it is possible that their low levels of λ1 reflect a more generalized regulatory defect affecting all λ gene expression. To evaluate this possibility it would be of interest to determine serum λ1 concentrations in other strains with unusually low λ2 (and λ3) serum levels, e.g. P/J, RIII/2J, C58/J, CE/J (70). It might also be useful to determine whether these other λ2 (λ3)-low strains have the same Kpn-resistant *Cλ1* site of SJL and BSVS mice. The retrovirus vector that is being used to study expression of λ1 genes [see above and (90)] may eventually prove valuable in determining the basis for defective λ1 expression in SJL and BSVS mice.

EXPANSION AND CONTRACTION OF λ GENES

Cλ Gene Segments in Inbred Mouse Strains

Sequence homologies are pronounced between the *Cλ2* and *Cλ3* gene segments and between the *Cλ1* and the unexpressed *Cλ4* segments (23–26). Likewise, the *J-C* introns of λ2 and λ3 are similar, and those of λ1 and λ4 are similar (25, 97). These relationships suggest that the four segments may have resulted from a relatively recent duplication in evolution of an ancestral

segment that contained two closely linked sequences, Cλx—Cλy, with Cλx the precursor of Cλ2, and Cλ3 and Cλy the precursors of Cλ1 and Cλ4 (25).

λ Gene Segments in Wild Mice

Another suggestion that the λ gene locus has undergone relatively rapid changes during evolution has emerged from interesting studies of wild mouse populations. Fragments of DNA from mice derived from such populations cross-hybridize strongly with probes for *Cλ* gene segments of the BALB/c mice (98, 99). This finding alone suggests pronounced sequence homology between *Cλ* sequences of inbred and feral mice. It also justifies the use of the BALB/c Cλ probes to estimate, by Southern blot hybridization assays, the number of *Cλ* gene segments in wild mouse populations. This number apparently varies from six to twelve in mice derived from two wild populations, *Mus musculus musculus* and *M. musculus domesticum* (98, 99). If inbred mice are descended from *M. musculus domesticum* (100, 101), several *Cλ* gene segments seem to have been deleted during the course of inbreeding. However, several distantly related species of the subgenus *Mus* appear to have only four *Cλ* genes, as in the inbred strains; if the inbred strains are descended from these other species then no change in the number of *Cλ* gene segments during inbreeding need be postulated. All of these considerations are, however, highly provisional, since they are based on the assumption that each positive band in a Southern blot represents a single gene segment. However, a single band could contain multiple segments and a single gene segment can give rise to more than one band.

Vλ gene segments. Southern blot analyses with probes for *Vλ* segments of BALB/c mice show no differences among the inbred mouse strains that have been examined. All appear to have two *Vλ* gene segments on restriction fragments that do not differ in size among the strains [(23) B. Blomberg, personal communication]. However, there appear to be at least three *Vλ* segments in *M. musculus domesticus* (102), raising the possibility again that a loss of λ gene segments (in this case *V*) occurred during the inbreeding process.

It is possible that many repetitive sequences or an unusual amount of sequence homology is present in the chromosome that contains mouse λ genes [chromosome 16 (29)]. Such sequences could provide a large target for recombination between sister chromatids and increase opportunities for amplification and contraction in the number of *Cλ* and of *Vλ* gene segments. A rough correlation between the number of *Vλ* and *Cλ* gene segments in *Mus* generally supports the notion that the recombination unit includes a single *Vλ* and linked *Jλ-Cλ* gene segments, as in the BALB/c mouse (23–26, 97) (see Figure 4). However, the evidence for inclusion of a *Vλ*

segment in this unit is still tenuous, because the $V\lambda$ has not yet been physically linked to the $C\lambda$ gene segments.

$C\lambda$ Gene Segments in Humans

Serological studies and amino acid sequences of constant region peptides from human Bence-Jones proteins led to the first suggestion that there are multiple nonallelic forms of λ chains (103–107). Detailed amino acid sequences indicated that there are at least four nonallelic forms (i.e. isotypes) in humans, but the exact number has not been clear because of difficulty in distinguishing allelic from nonallelic variants in outbred populations. Recent studies of human DNA initiated with λ cDNA probes of mouse (BALB/c) origin, have led to the isolation of three $C\lambda$ gene segments that correspond to known human λ isotypes (108); three additional $C\lambda$ gene segments have been identified but not yet sequenced. All six reside in tandem in a 50 kb segment on chromosome 22 (108). As yet unlinked to this cluster are three additional λ-like segments and several DNA fragments that cross-hybridize weakly with human and mouse λ probes. How many of these genes are functional is not clear, but an unusual λ pseudogene, identified on a different chromosome, has the sequence expected of cDNA for a processed RNA molecule, i.e. one from which the noncoding sequence between the J and C gene segments has been excised (109).

Aside from the multiplicity of $C\lambda$ gene segments, the extent to which the organization of human and mouse λ gene segments are alike is still not clear. It is not known, for instance, if each human $C\lambda$ gene segment is associated with its own $J\lambda$ gene segment, or if the $J\lambda$ segments in the human genome are clustered (as in the H and K gene families). The number of $V\lambda$ gene segments is also unknown, but it is expected to be substantially greater in man than in inbred mice, because the proportion of human Igs with λ chains are almost 10-times greater than mouse Igs with these chains (about 40% vs. about 5%).

CONCLUDING REMARKS

This review emphasizes three properties of λ chains and genes in inbred mice: their organization and diversity, the relationships between their primary structure and ligand-binding activity, and the different frequencies with which the several isotypic forms are found in serum Ig, spleen B cells, and antibodies to various haptenic groups. The simplicity of this Ig gene family has made it possible to attempt a quantitative assessment of the extent to which various mechanisms contribute Ig V region sequence diversity. For λ1 chains, somatic mutation is clearly the great diversifier:

V/J junctional amino acid substitutions, arising from imprecision in gene segment assembly, are responsible for structural variations that are few in number but large in impact on ligand-binding activity: However, V-J combinatorial variation due to recombination of different V and J gene segments is virtually nonexistent, at least when the individual isotypes are considered.

Several provocative regulatory problems have emerged from studies of these chains. One is the much greater frequency of $\lambda 1$ than $\lambda 2$ or $\lambda 3$ chains. The difference between $\lambda 1$ and $\lambda 3$ is especially intriguing because both use the same V gene segment. Another regulatory problem of interest is the defective expression of $\lambda 1$ (and also the other λ chain isotypes) in SJL mice. Despite the simplicity of the λ-gene family a solution to these regulatory problems is not yet at hand. It is possible, nonetheless, that further analysis of these chains and genes will contribute as much to our understanding of regulation problems as they have to our understanding of the sequence organization and diversity of Ig chains and genes in general.

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Annual Review of Immunology
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CONTENTS